Breeding Tubercles, Papillomatosis and Dominance Behaviour of Male Roach (Rutilus rutilus) During the Spawning Period

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Abstract

We studied the relationship between the breeding tubercle ornamentation (i.e. skin roughness) and male pre-spawning dominance and courtship behaviour in roach (Rutilus rutilus) within an experimental laboratory system. Sexually mature fish were caught during their migration to their spawning pond and their behaviours were studied in an artificial spawning arena. Males behaved naturally both in terms of male–male interactions and attempts to achieve spawnings. Males having many, large breeding tubercles (i.e. rough skin) were significantly more often dominant in our dyadic trials than those with smooth skin. The dominant male in the trial exhibited a more active courtship behaviour than its subordinate rival. Papilloma skin disease did not affect the dominance rank. As a result of the relationship between skin roughness and male dominance, breeding tubercles may be used by the females as a cue for choosing a high-quality mate in a roach lek. Thus, breeding tubercles might offer a workable tool for examination of sexual selection among cyprinids.

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Introduction

Male–male competition may be an important factor for determining reproductive success in lek-like breeding systems (Andersson 1994). Various reproductive strategies have been found in fish (Oliveira & Almada 1998; Taborsky 2001). Bourgeois (i.e. dominant territory owner) and parasitic roles of males in spawning are alternative mating tactics. These are not exclusive, however, as bourgeois males can also show opportunistic behaviour (Taborsky 1998). In group or broadcast spawners, the behavioural patterns are not clear, but some subtle levels of monopolization may occur (Wedekind 1996).
The roach (*Rutilus rutilus*) is a cyprinid fish spawning in spring in large groups in relatively shallow waters. Roach spawn on stones and vegetation, and the breeding sites can vary considerably between populations and localities (Mills 1981, 1991; Diamond 1985; Gillet & Dubois 1995). This species often migrates to favoured spawning sites (L’Abee-Lund & Vollestad 1985; Mills 1991; Kestemont et al. 1999) such as littoral waters, bays, creeks and small ponds, which are warm early in the spring. Roach breed using a lek-like mating system in which males can have bourgeois and/or parasitic roles (Wedekind 1996). Direct observations of spawning events are uncommon because of poor visibility in field conditions. However, in previous studies, females chose males having some kind of territory, and multi-male fertilization was common (Diamond 1985; Wedekind 1996). Female roach are often larger than males (Spivak et al. 1979; Diamond 1985; Vollestad & L’Abee-Lund 1987). Females have strong individual preferences with respect to males, and mate choice is not random (Wedekind 1996). Roach show no parental care for their offspring, and females obtain only gametes from the males. Thus, roach mating may be a typical non-resource-based lek-mating system as defined by Hoglund & Alatalo (1995). However, according to our observations, roach spawning is not as tightly place-bonded as a traditional bird lek.

Breeding tubercles are keratin-based epidermal nodules, which are found in at least 15 families of fishes in four orders. The tubercles are found most often on males and are induced by several sex hormones (see review by Wiley & Collette 1970), especially testosterone and 11-ketotestosterone (Kortet et al. 2003). The development of breeding tubercles may take weeks rather than days and they are shed shortly after spawning (Wiley & Collette 1970). The role and evolution of breeding tubercles are not well known. Breeding tubercles may be used for conspecific recognition (Vladykov et al. 1985), or for protection against mechanical injuries (Ahnelt & Keckeis 1994). Muller & Ward (1995) suggested that breeding tubercles may be used as weapons in intense pre-spawning male behaviour (defence of nests and territories). However, Wiley & Collette (1970) proposed that tubercles originally evolved to allow breeding individuals to maintain close contact during spawning as a means to ensure fertilization of the eggs. Breeding tubercles may also act as hydrodynamic or tactile stimulators of females during courtship. In roach, breeding tubercles are presumed to give females detailed information about a male’s parasite load (Wedekind 1992; Kortet & Taskinen 2004) and parasite resistance (Taskinen & Kortet 2002; Kortet et al. 2004), and to act as a sexual ornament indicating his quality. According to our observations, there is remarkable variation in the appearance of the breeding tubercles between and within wild roach populations but, to our knowledge, no behavioural studies have been conducted to examine the signalling role of breeding tubercles as either a sexual ornament or a badge of status.

Epidermal papillomatosis is a stress-induced, mainly viral disease (Mellergaard & Nielsen 1995; Premdas et al. 1995, 2001). Sexual maturity and reproduction cause hormonal and physiological stress (e.g. Pottinger et al. 1995), which may increase the risk of disease. Many roach, especially males, in populations within our study area, suffer from papilloma skin disease during spawning (Kortet
et al. 2002). For example, the prevalence of this disease among males in 1999 and 2000 was 10% in a Lake Konnevesi population (Kortet et al. 2002). As papillomatosis is a stress-induced and hormone-based disease (Premdas et al. 2001; Kortet et al. 2003) occurring during spawning, its presence might influence the behaviour of breeding fish, and especially female choice of mates.

The aim of our study was to test whether breeding tubercles could be related to male dominance status by examining courtship and mating behaviours in pre-spawning events. Dominance may help males to improve their fertilization success, as there is competition for spawning sites within a roach lek (Wedekind 1996). Because development of breeding tubercles is contributed by androgens, we predicted that they should be related to the dominance status of a male. Parasitism has been suspected to be important in intersexual selection but parasites and diseases also play a role in intrasexual selection (Hamilton & Poulin 1995; Barber 2002). Therefore, we also studied the incidence of epidermal papillomatosis in males and its effect on their behaviour patterns. We predicted that papillomatosis would be negatively related to male dominance status as well as to the incidence of courting behaviour. We also estimated the frequency of indications of parasitic spawning behaviours by subordinate male roach in our experimental trials to yield additional new information about roach spawning, in general.

**Methods**

**Study Site and Animal Collection**

Sexually mature fish were caught using dip nets from a creek (62°15’N, 26°26’E), as they migrated from Lake Konnevesi to their spawning site, Kopru Pond, located 70 km north-east from the city of Jyväskylä, Finland. Collections were made on May 24, 25 and 28, 2000. Water temperature was 15°C in the creek and 10°C in Lake Konnevesi. After collection, we transported the fish in tubs to the laboratory at the Konnevesi Research Station. In the wild, decreasing water temperature may temporarily interrupt migration and spawning (Vøllestad & L’Abeé-Lund 1987). In northern boreal waterways, creek water temperature can be quite variable and fish often experience quick temperature changes when they migrate from cold lakes to warmer creeks. Therefore, in the laboratory, the fish were maintained at 11°C in single-sex groups in circular 1500 l tanks to keep their spawning stage stable prior to behavioural tests. Fish were fed daily with commercial dry pellets. Behavioural experiments began on the day after collection and continued for 9 d.

**Test Procedure**

Two males of similar length (Table 1) and one female were moved by dip net to a spawning arena (length 100 × width 65 cm, depth 65 cm), located in a stream tank (length 600 × width 65 cm) with slowly circulating water. The breeding
tubercle ornamentation of males (roughness of body sides and operculum) was estimated by hand twice – once before and once after experiments (Taskinen & Kortet 2002). Males were ranked into three groups according to their ornamentation: (1) no breeding tubercles or very few or only very small tubercles, smooth skin, (2) breeding tubercles clearly present, slightly or moderately rough skin, and (3) rough skin with many large tubercles. One person ranked all the fish. In order to verify the repeatability of this ranking method, another set of fish \( n = 72 \) was ranked by several persons. Repeatability was tested using a kappa measure of agreement. The agreement between measurers was highly significant (kappa value = 0.916; \( p < 0.001 \)). All the males studied, including the ones with no breeding tubercles, were sexually mature and ready to spawn. The maturity of the males was verified by examining their gonads post-mortem.

The spawning arena was isolated with metal netting and equipped with one spawning site composed of aquatic moss \((Fontinalis\ sp.)\), willow roots \((Salix\ sp.)\) and stones. Light conditions in the laboratory followed a natural rhythm (4 h dark/20 h light) and the water temperature in the stream tank was kept between 18 and 20°C. We simultaneously used two identical stream tanks with one spawning arena in each. A video recorder and infrared light sources were placed on the side of each arena, for observation of behavioural variables under low illumination, to avoid disturbing the fish. The experimental males were marked

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BT, breeding tubercle ornamentation group; A, age; L, length (mm); C, condition factor; P, papillomatosis (1, healthy; 2, diseased); Co, courting behaviours; D, dominance-indicating behaviours.

Table 1: The fish used in the experimental trials
with a 5-mm spot of neutral red dye (Merck, Darmstadt, Germany) on their dorsal surface (either in front of or behind the dorsal fin) by removing one fish scale and painting the mark on the skin. According to our preliminary tests these markings lasted for 2 d. Ten sexually mature females and five sexually mature males were placed in the stream tank channel upstream from the spawning arena to stimulate prespawning behaviours in the experimental fish.

Experimental fish were observed, and after the males began to show identifiable dominance or courtship behaviours, which usually happened within 15 min, their actions and behavioural patterns were recorded on videotape for 3 h. The ornamentation of the males was not known to those who analysed the videotapes. We analysed the videotapes at a later date and monitored male courtship (quivering and butting or chasing the female) and dominance behaviour (aggressive butting and chasing the other male). Butting against the female was often slightly more gentle than butting against the rival male. In this butting, the male bumped the female physically with its forehead. Continuous butting or chasing was given only one count. All the continuous buttings and chasings lasted <10 s. As a result of the lek-like spawning of roach, where females visit loose territories defended by males (Diamond 1985; Wedekind 1996), we interpreted aggressive male–male interactions as indicators of dominance in the pre-spawning hierarchy. These kind of antagonistic interactions are typical for male roach only during the short spawning period (Wedekind 1996). In addition, we assumed that, in our trials, all behaviours targeted towards the female can be interpreted as courtship in a very general sense, because in the mating system of roach it is the female that ultimately makes the choice. Quivering, butting and chasing females have been described as male behaviours to obtain matings (Diamond 1985; Wedekind 1996). All these courting behaviours were pooled to obtain one variable of 'courtship'. Likewise, we pooled all dominance-reflecting behaviours to one variable of 'dominance' by adding those behaviour counts together. The male having a higher frequency of dominance behaviours was ranked as the dominant individual in each trial. Indications of parasitic spawning actions, during which both males tried to achieve spawning with the female simultaneously, were noted whenever they occurred during an experiment. In those cases, both males chased the female and quivered vigorously, most often with the subordinate male behind the dominant. However, the efforts of these males to obtain matings were quite similar. In roach, parasitic spawning is probably an opportunistic behaviour performed by males using various strategies.

The repeatability of our counts, R (see Krebs 1989), of different behavioural observations from videotape was high ($R = 0.943, F_{19,20} = 33.596, p < 0.001$). The experiment was performed 18 times with 36 different males and nine females. We assumed that male dominance was not strongly affected by the specific female used in a trial. Therefore, each female was used twice.

After the experiment, all fish were killed by a sharp blow to the head, and measured. Using weight without intestinal organs (W, g) and total length (L, cm) measurements, an index of each fish’s condition (condition factor, K) was
calculated using the equation \( K = \text{constant} \times \frac{W}{L^b} \), where \( b \) is the slope of a regression of \( \log_{10}(W) \) on \( \log_{10}(L) \) (Bolger & Connolly 1989). No change in the ornamentation of males was found during the trials. We also noted the presence of papilloma disease (categorizing fishes as healthy or diseased, based on visual observation) and determined the age of the fish from scales taken between the pectoral fin and the lateral line. This method for age determination has previously been used for roach (e.g. Horppila 1994).

Statistical analyses were performed with SPSS 7.5 (SPSS Inc. Chicago, IL, USA). The parameters that were untenable for use in ANOVA were log-transformed (length and age).

**Results**

Fish size varied within age classes [mean length (mm; \( \pm \) SD) values to males: 5-yr-old: 151 \( \pm \) 0, \( n = 2 \); 6-yr-old: 156.8 \( \pm \) 10.7, \( n = 12 \); 7-yr-old: 167.9 \( \pm \) 10.4, \( n = 17 \); 8-yr-old: 196.0 \( \pm \) 5.6, \( n = 4 \); and 9-yr-old: 190, \( n = 1 \) (Table 1).

Males displayed clear pre-spawning behaviours: dominance (butting and chasing the other male) and courtship (quivering, butting and chasing the female) in the presence of a female (Table 1), despite the fact that females did not release eggs into water during our experiment. Indications of simultaneous parasitic spawning behaviour (or scramble competition), where the subordinate male tried to achieve spawnings from the female physically at the same time as the dominant male, occurred in 15 of 18 experiments, and it occurred in all ornamentation (skin roughness) groups. In five cases, we observed aggressive behaviour of females towards a courting male (chasing him away). As the females were used twice in our trials, we tested if there was any difference in the overall behavioural activity of males between the first and the second use of the female. However, no difference in the dominance (Wilcoxon signed ranks test: \( Z = -1.103, n = 9, p = 0.270 \)), or courtship (Wilcoxon signed ranks test: \( Z = -1.125, n = 9, p = 0.260 \)) scores of males could be found between the first and second trials of the same female, indicating that the use of the same female two times did not affect the behavioural activity of the males.

Breeding tubercle ornamentation was significantly related to dominance status (chi-square test for frequency table: \( \chi^2 = 9.365, \text{d.f.} = 2, p = 0.009 \)) (Fig. 1). In addition, courtship behaviour was positively related to dominance status (paired t-test: \( t = -2.456, n = 18, p = 0.025 \)) (Fig. 2), unlike condition factor (Wilcoxon signed ranks test: \( Z = -0.924, n = 18, p = 0.355 \)), age (Wilcoxon signed ranks test: \( Z = -0.749, n = 18, p = 0.454 \)), length (Wilcoxon signed ranks test: \( Z = -0.915, n = 18, p = 0.360 \)) or papilloma skin disease (Wilcoxon signed ranks test: \( Z = 0, n = 18, p = 1.000 \)).

There was no relationship between papilloma disease and fish length (ANOVA: \( F_{1,34} = 3.210, p = 0.082 \)), condition factor (ANOVA: \( F_{1,34} = 2.733, p = 0.108 \)) or age (ANOVA: \( F_{1,34} = 1.368, p = 0.250 \)). Breeding tubercle ornamentation was not related to condition factor (ANOVA: \( F_{2,33} = 0.178, p = 0.185 \)), length (ANOVA: \( F_{2,33} = 0.869, p = 0.429 \)), age of the fish (ANOVA: \( F_{2,33} = 0.599, p = 0.555 \)) or
papillomatosis (Pearson: $\chi^2 = 1.609$, n = 36, p = 0.447). Moreover, breeding tubercle ornamentation was not related to courtship activity (ANOVA: $F_{1,34} = 0.420$, p = 0.660).

Fig. 1: Ornamentation rank of subordinate and dominant male roach used in the experimental trials. (1) No breeding tubercles or very few or only very small tubercles, smooth skin; (2) breeding tubercles clearly present, slightly or moderately rough skin; and (3) rough skin with many large tubercles.

Fig. 2: Courtship activity of subordinate and dominant male roach used in the experimental trials. Courtship activity was measured as counts of quivering, butting and chasing the female. Vertical bars represent ±1 standard error of the mean.
Discussion

We found a relationship between breeding tubercles (skin roughness) and dominance behaviour, indicating that breeding tubercles may have a role as a status badge or as a signal of quality in intrasexual selection of roach. Neither the condition factor, nor the age of males was related to dominance, probably because male pairs in our study were size-matched. By controlling the size of the fish, however, we could study the relations between breeding tubercles and fish prespawning behaviours in our experimental system. In brown trout (*Salmo trutta*), dominance rank previously has been found to increase with age and size, which in turn influence male spawning success (Petersson & Järvi 1997). As no studies have been carried out in roach on the effect of size on dominance or courting behaviour, we do not know how significant the effect of size is in natural spawning aggregations.

Our measure of dominance, the count of dominance-indicating behaviours, does not take into account the intensity of these behaviours. By excluding the assessment of the intensity of each behaviour we likely have lost some additional information. However, by counting the number of behaviours, we have gained simplicity and, probably, higher reliability of our measure. It is problematic to rank different components of dominance by trying to evaluate which ones are more important, e.g. whether a slightly longer chase is more important than a few additional butttings. Therefore, in our dominance behaviour score we gave equal importance to various dominance-indicating behaviours. We think that the total counts of dominance-indicating behaviours gives a reliable picture of the prespawning dominance status (i.e. aggressiveness) of the fish in general. Similarly, our interpretation of courtship might be problematic, because it is possible that male quivering, butting or chasing the female are not pure courtship behaviours but may also reveal the male’s attempt to force a mating or to repel females that are not prepared to spawn. However, as these kinds of behaviours of male roach during spawning have previously been interpreted as males’ ways to coax females to mate (i.e. courtship) (Diamond 1985; Wedekind 1996), we adopted this interpretation here.

Breeding tubercles are found among several fish species (Wiley & Collette 1970), but their role and function are still uncertain. Our results provide the first evidence for the possibility that breeding tubercles serve as signals in the pre-spawning behaviour of fish. We think that the signal may be tactile or hydrodynamic, rather than primarily visual. As dominant males had rougher skin than subordinates, tubercles may function as an intrasexual ornament or as a status badge. Dominance and the expression of skin tubercles may share the same causal factor, e.g. androgens. Indeed, Kortet et al. (2003) showed that elaborated breeding tubercle ornamentation is associated with high circulating levels of testosterone and 11-ketotestosterone. Thus, the badge of status, in this case, breeding tubercles, could signal the quality of its bearer. Furthermore, as breeding tubercles have been shown to indicate parasitic load (Wedekind 1992; Kortet & Taskinen 2004), parasite resistance (Taskinen & Kortet 2002; Kortet
et al. 2004) and offspring survival (Wedekind et al. 2001), it seems probable that they may also function as an ornament of both inter- and intrasexual selection. This does not exclude the possible role of tubercles as protection against physical injury during spawning (Ahnelt & Keckeis 1994). A status badge may signal good quality to females as well as to males (Berglund et al. 1996) and the present results suggest that this may be the case for breeding tubercles in roach. In the case of secondary sexual traits, male contests very often favour the same signal traits that females prefer (Andersson 1994; Berglund et al. 1996). There are no studies in roach of how courtship and male dominance are related to fitness. In salmon, males showing less quivering and courting behaviour also had a lower reproductive success (Jonsson 1997).

Papillomatosis may be harmful by increasing risk of secondary fungal or bacterial infections, or of predation as suggested by Kortet et al. 2002. Papillomatosis induced by *Herpesvirus cyprini* has been found to be virulent for carp fry (Sano et al. 1991). Interestingly, in the present study, the papilloma disease did not affect the dominance status of the male. The diseased fish in our trials were able to carry the papillomatosis load and at the same time express their quality with the number and size of breeding tubercles (cf. Zahavi 1975). Likewise, neither Hamilton & Poulin (1995) nor Barber (2002) could find any connection in fishes between parasite load and male dominance.

In our study, indications of simultaneous parasitic spawning attempts were very common. It is difficult to define clear alternative reproductive tactics in group-spawning fish like roach (see Taborsky 2001). However, we tried to evaluate dominance of males that tried to achieve spawning simultaneously. In group spawners, this kind of behaviour can also be interpreted as scramble competition as defined by Taborsky (2001). The high frequency of simultaneous parasitic spawning actions in roach (Svärdsøn 1951; Diamond 1985; Wedekind 1996) indicates that sperm competition, operating with, e.g. ejaculation quality might be an important intrasexual selective force (Taborsky 1998). Male roach do not offer clear shelters, i.e. nests or burrows, where they can spawn alone with the female, which increases the possibility of simultaneous parasitic spawning. Therefore, a high dominance rank (i.e. frequency of aggressive behaviours) of a male and the ability to keep other males away from the female probably increases fertilization success. The ability to keep harassing rival males at a distance from the female has been found to be the key variable for the breeding success of males in lekking birds (Alatalo et al. 1991).

Non-random patterns in spawning behaviour have been observed in group-spawning fish (Taborsky 1998). Thus, the correlation found between male dominance status and the breeding tubercle ornamentation in roach may provide new insights for studies of lek-mating in fishes. However, more experimental studies are needed, possibly with manipulation of androgens, to understand the signalling role of the breeding tubercles in fishes.
Acknowledgements

Our collections and experiments were conducted with the permission from the Ethical Committee for Animal Research of the University of Jyväskylä (permit LS-16/00). We are grateful to V. Kupari and C. Madden for assistance in the field and laboratory and Konnevesi Research Station for providing good facilities for the experiments. We thank E.T. Valtonen, S. Kuukasjärvi, M. Runcie, J. Pirhonen, E. Knott, R. Jones, J.D. Roberts, M. Taborsky, A. Hedrick and anonymous reviewers for helpful comments on earlier versions of the manuscript. We would also like to thank A. Lyra for assistance with statistical analyses and E. Rimaila-Pärmänen from the Veterinary Research Institute of Finland (EELA) for the identification of papilloma disease. We thank the Emil Aaltonen Foundation for financial support to R.K. and the Finnish Academy for support to H.Y.

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Breeding Tubercles Indicate Dominance in Male Roach


Received: November 15, 2002

Resubmitted: March 11, 2003

Initial acceptance: August 28, 2003

Final acceptance: April 22, 2004 (M. Taborsky)